anatomic regions and within any given tissue.

Steinert et al. also interrogate the second assumption: that T_{RM} faithfully express CD69 and/or CD103 and are not accessible to intravascular antibody. Using parabiotic pairs of congenic mice, which were surgically joined to establish a shared blood circulation, the authors discover that a sizeable fraction of T_{RM} express neither CD69 nor CD103, and some T_{RM}, especially in the kidney and liver, actually appear to reside within the intravascular space.

These findings have implications for how immunologists think about T cell surveillance of tissues, particularly with regard to T_{RM}. For example, in the FRT, isolation-based methods had estimated that there is one T_{RM} for every ~20,000 nucleated cells, while tissue microscopy performed by Steinert et al. reveals that there is one T_{RM} for every ~300 nucleated cells. Assuming that T_{RM} within the FRT scan cells at a similar rate to those in the skin (Ariotti et al., 2012), isolation-based methods project that T_{RM} would require ~1 month to scan every cell in the FRT. In contrast, the tissue microscopy data imply that T_{RM} scan the FRT in its entirety within ~12 hr, an estimate that is much more consistent with the reported effectiveness of T_{RM} to protect non-lymphoid tissues (Mackay et al., 2012).

Steinert and colleagues thus provide a much-needed reality check for immunologists. Their findings will have to be taken into account when evaluating immune responses to vaccines and pathogens, and it will be important to determine their impact on our understanding of allergic and autoimmune diseases, as well as immuno-oncology.

Even though 80 years have passed since the *Literary Digest* fiasco, this study provides a stern reminder that sample bias is not a fiction of the past but remains to this day a fact to be reckoned with—by scientists and voters alike.

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Shedding Light on Glioma Growth

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Cancer is known for opportunistically utilizing resources from its surroundings for its own growth and survival. In this issue of *Cell*, Venkatesh et al. demonstrate that this also occurs in the brain, identifying neuronal activity-induced secretion of neuroligin-3 as a novel mechanism promoting glioma proliferation.

Cancer is notorious for hijacking normal biological processes to promote tumor cell survival, migration, and proliferation. Cancer cells release angiogenic factors that promote blood vessel formation to support their own survival and upregulate molecules normally expressed by healthy cells to evade immune detection. In their recent study, Venkatesh et al. (2015) reveal that cancer cells also take advantage of neuronal activity, the most essential aspect of brain function, in order to proliferate. The authors demonstrate that optogenetic stimulation of neurons can promote the growth of human high-grade gliomas (HGGs) by inducing the secretion of mitogenic factors.

This study was initiated following the discovery that neuronal activity stimulates the proliferation of oligodendrocyte precursor cells (OPCs) and neuronal precursor cells (NPCs) in vivo (Gibson et al., 2014), cells that can give rise to gliomas (Cuddapah et al., 2014). Both studies utilized optogenetic strategies to increase neuronal activity by stimulating channelrhodopsin-expressing neurons with blue light (Figure 1A). This approach enables the activation of subsets of neurons in defined circuits in a physiological manner and allows for comparisons between different circuits or regions from within the same brain. Importantly, this method

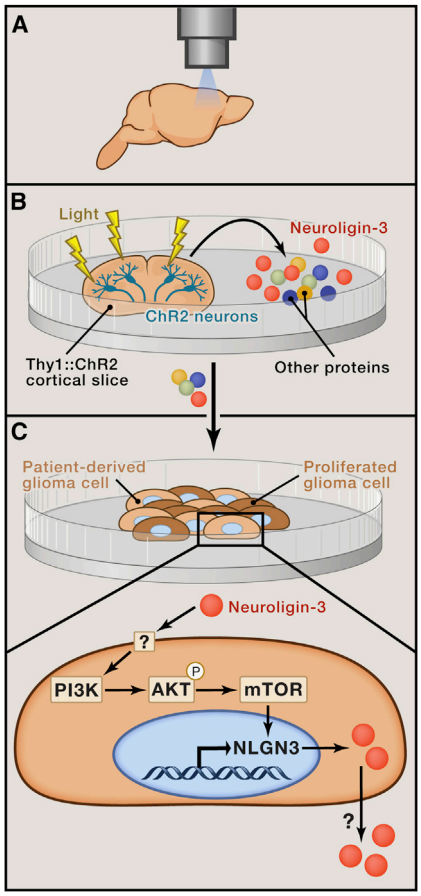

Figure 1. Optogenetic Techniques Reveal that Activity-Induced Secretion of Neuroligin-3 Promotes Glioma Growth

(A) In vivo optogenetic stimulation of Thy1::ChR2 premotor cortex promotes the proliferation of xenografted glioblastoma cells.

(B) In vitro optogenetic stimulation of Thy1::ChR2 cortical slices leads to activity-regulated secretion of factors into the media.

(C) Conditioned medium from stimulated cortical slices induces growth of patient-derived glioma cells in vitro. Venkatesh et al. identify secreted neuroligin-3 as their primary candidate mitogen and propose a downstream signaling pathway involving PI3K and mTOR.

can be used to stimulate the brains of awake, behaving animals.

Venkatesh et al. put this technique to use in their orthotopic xenograft model of pediatric HGG. To create this model, the authors xenografted cells cultured from a biopsy of frontal cortex glioblastoma from a 15-year-old patient into the premotor cortex of immunodeficient mice that had been crossed to the Thy1::ChR2 line, which would allow for activation of neural tissue surrounding the xenografted cells. Just as in non-pathological experiments with OPCs and NPCs, optogenetic stimulation of neuronal activity using blue light induced proliferation of xenografted pediatric HGG cells. A single stimulation was sufficient to induce proliferation; however, repetitive stimulation over the course of a week further increased tumor cell burden.

The authors next moved to an in vitro system to investigate the mechanism underlying neuronal activity-induced glioma proliferation (Figure 1B). After determining that conditioned medium collected from optogenetically stimulated Thy1::ChR2 cortical slices could induce the proliferation of a variety of different patient-derived HGG cell cultures (Figure 1C), Venkatesh et al. sought to identify the secreted signal responsible. Glioma cells express ion channels and neurotransmitter receptors and are sensitive to calcium, and, therefore, could proliferate in response to a variety of secreted signals (Cuddapah et al., 2014). Using mass spectrometry, the authors identified secreted neuroligin-3 in the cortical slice-conditioned medium as their primary candidate mitogen and confirmed its ability to induce the proliferation of multiple types of HGG using a recombinant protein. Importantly, the neuroligin-3 found in the conditioned medium contained only the ectodomain, suggesting that it is cleaved in a similar manner to known family member neuroligin-1 (Peixoto et al., 2012; Suzuki et al., 2012).

To understand how neuroligin-3 could exert this mitogenic effect, the authors performed RNA sequencing followed by western blot analysis on cultured glioma cells that had been treated with either light-exposed WT or Thy1::ChR2-conditioned medium. They determined that neuronal activity-regulated secretion of neuroligin-3 promoted glioma cell proliferation through activation of the PI3K-mTOR pathway (Figure 1C). Interestingly, this pathway activated both transcription and translation of neuroligin-3 in glioma cells, suggesting a feedforward signaling loop. Increased neuroligin-3 expression by tumor cells may indeed be pathogenic, as the authors found an inverse relationship between adult glioblastoma neuroligin-3 mRNA expression and patient survival upon analyzing data from The Cancer Genome Atlas. On average, patients categorized as having high levels of glioblastoma neuroligin-3 expression had a lifespan that was 5 months shorter than that of patients with low expression of neuroligin-3. This effect was specific, as there was no association between expression of neuroligin-2, which does not induce glioma cell proliferation in vitro, and patient survival.

The finding that neuronal activity promotes glioma proliferation raises a number of interesting questions. Previous studies have found that cancer cells secrete glutamate, which may stimulate their own proliferation, and that glutamate secretion may also be linked to the epilepsy developed by many glioma patients (Buckingham et al., 2011). Given this new link between neuronal activity and glioma growth, one question that arises is whether tumor-associated epilepsy serves as another type of feedforward mechanism to support further glioma proliferation. Additionally, this link may also shed light on studies that demonstrate an increased likelihood of brain tumor development in patients who have been treated for epilepsy, which is not very well understood (Khan et al., 2011).

The discovery of neuroligin-3 as a potential mitogen is also unexpected, given its role as a postsynaptic adhesion molecule required for normal synaptic function and its implication as a disease gene in autism (Südhof, 2008). This study suggests that neuroligin-3 may play other roles in non-neuronal cells and that secreted neuroligin-3 may regulate its own transcription. Additional work is necessary to understand the mechanism outlined in this study, including the cellular origin of secreted neuroligin-3, the activity-dependent neurolign-3 cleavage mechanism, the recruitment of downstream PI3K, and the function of tumor-derived neuroligin-3. Some insight into neuroligin-3 cleavage may be gleaned from studies that have examined neuroligin-1, which has been shown to exhibit activity-dependent cleavage and secretion of its ectodomain (Peixoto et al., 2012; Suzuki et al., 2012). These findings also highlight the need to investigate the role of secreted neuroligin-3 in the healthy brain and whether the feedforward pathway is utilized for growth of healthy OPCs and NPCs or only arises in glioma cells.

Venkatesh et al. provide invaluable insight into HGG, revealing not only a greater mechanistic understanding of the regulation of glioma growth, but also a potential therapeutic target in neuroligin-3. Their observations that neuronal activity promotes the proliferation of multiple glioma types and that neuroligin-3 is mutated in a variety of different types of cancers, combined with recent studies implicating autonomic innervation with cancer progression in other systems (Magnon et al., 2013; Zhao et al., 2014), suggest that this mechanism may be broadly applicable to many cancers.

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Rods Feed Cones to Keep them Alive

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Cone photoreceptors, responsible for high-resolution and color vision, progressively degenerate following the death of rod photoreceptors in the blinding disease retinitis pigmentosa. Aït-Ali et al. describe a molecular mechanism by which RdCVF, a factor normally released by rods, controls glucose entry into cones, enhancing their survival.

The retina is a highly sophisticated biological computer that captures an image with its photoreceptors and extracts different visual features to describe the visual scene to higher brain centers in simple and compact terms. Although photoreceptors, the rods and cones, are only two out of the sixty retinal cell types, they are exceptionally important: all image-forming vision depends on their proper function. Despite the fact that rods outnumber cones 20 to 1, human vision is mostly based on cones. Rods are distributed at the periphery of the retina and are the photosensors for low light levels. Cones are concentrated in the center of the retina and work at higher light levels. Since cones are necessary for the high-resolution color vision that enables us to read, recognize faces, and enjoy the colorful world, in the modern world we surround ourselves with enough light to turn on the cones. Most of us spend little time in conditions where photons are scarce and, therefore, our dependence on rod function is minor. A study presented in this issue of *Cell* offers key insight into the interdependence of rods and cones, and how it is disrupted in the genetic disorder retinitis pigmentosa (Aït-Ali et al., 2015)

The genes involved in retinitis pigmentosa are primarily expressed only in rods and are important for their function (Hartong et al., 2006). If humans rely mostly on cone vision, why is this disease so severe? The reason stems from the fact that rods and cones are dependent on each other. When rods are dysfunctional but alive, as in another genetic disease called stationary night blindness, cones are functional. Indeed, patients with stationary night blindness are capable of living an almost normal life. However, when rods die, as happens in retinitis pigmentosa, cones sense this loss and react to it. This reaction is devastating. First, cones lose their outer segments, which serve as light detectors, causing patients to become blind. Second, on a longer timescale, the other parts of the cones progressively degenerate.

Due to the importance of cones for human vision, and their dependence on rods, two fundamental questions in retinitis pigmentosa research are why and how do cones react to rod death and how can we prevent cones from degenerating?. There have been several important insights in recent years. One of these insights, originating in José-Alain Sahel's laboratory, came from the logic that if rods are necessary for cone